Identification of Transcribed Differentiating Genes in *Brucella abortus*, *B.melitensis and B.suis*

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1 Introduction.

Design of diagnostics based on microarray technology inherently involves a multiple genome comparison. Unique regions must be unequivocally identified; this process can not rely solely on comparison of annotated gene sequences, especially when annotation is tentative or incomplete. A three-way comparison of published and annotated genomes of *Brucella. melitensis* and *B. suis,* and a draft sequence of the *B. abortus* genome identified a group of unique known and hypothetical gene coding sequences in these species [1]. The comparison was performed using a prototype of the GenoMosaic toolkit [2]. Although only *B. suis* was found to have a significant number of unique genes, patterns of genes that exist in only two out of three genomes were also identified. These patterns will discriminate unambiguously between *B. suis*, B. *melitensis*, and B. *abortus*, and are important for explaining the differences in virulence and host specificity of the three *Brucella* spp. The existence and *in vitro* transcription behavior of the differentiating genes were confirmed by PCR.

Brucella is a facultative intracellular pathogen with approximately 3 Mb genome, split between the two chromosomes the size of 1.85 Mb and 1.35 Mb. Human brucellosis is quite common but often not diagnosed. There are six recognized *Brucella* species that differ in their host preference. *B. abortus* preferentially infects cattle, *B. melitensis* infects sheep and goats, and *B. suis* infects pigs. All three of these species and *B. canis* can infect humans, although *B. melitensis* is associated with the most serious human infections. The *Brucellae* are grouped with the α-proteobacteria and are related to other cell-associated parasites of plants and animals [3]. The true pattern of *Brucella* intracellular survival and proliferation, and the reasons for the different virulence patterns among the species are not conclusively known.

2 Experiment and Methods.

Reverse transcriptase (RT-PCR) analyses were performed for identified unique and differential regions of three *Brucella* spp.**:** ***B. abortus*, *B.*** *melitensis* and ***B. suis,*** to determine whether the identified differentiating genes are transcribed *in vivo*. To compensate for the absence of completed annotation in *B.abortus,* the identified differentiating open reading frames from *B. melitensis* and *B. suis* were used to develop the RT-PCR primers. The same primers were used to interrogate each of the three genomes, testing for the existence and expression of these genes in *B. Abortus*. Patterns of identified unique and differentiating genes that exist in only two out of three genomes were

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confirmed by PCR. Both PCR and RT-PCR reactions were performed for 104 unique genes or partial differential genes detected in the three genomes by whole genome sequence comparison[2]. 23 unique genes, and 79 differential genes common between the two of the three species were tested for transcription. Table 1 summarizes transcripts detected for genes in each differentiating sequence island.

Genomic	*Brucella suis*			*Brucella melitensis*			*Brucella abortus*		
location	Predicted	Observed	NB	Predicted	Observed	NB	Predicted	Observed	NB
S1	4	4	0	1	1	0	0	0	0
S2	18	17	1	0	0	0	0	0	0
M1	0	0	0	1	1	0	0	0	0
A	0	0	0	0	0	0	1	1	0
SM1	1	1	0	2	2	0	0	0	0
SM2	25	16	9	24	6	18	0	0	0
SA1	11	3	8	0	0	0	9	7	2
SA2	11	7	4	0	0	0	11	6	5
MA1	0	0	0	30	21	9	26	23	3

Table 1: Summary of RT-PCR results for differential ORFs.
A – *B.abortus*, M – *B.melitensis*, S – *B.suis*, 1 – chromosome I and 2 – chromosome 2 and NB – no band.

Several groups of genes with potential significance for virulence were detected as differentials and shown to be transcribed. Some of these genes were likely of phage or plasmid origin, suggesting possible mechanisms for their appearance as differentials.

3 Conclusions.

In three very closely related pathogen genomes, systematic genome-wide comparison was used to identify targets for a diagnostic microarray. RT-PCR tests confirmed computational predictions. PCR amplification of the genomic DNA confirmed the presence of the predicted amplicon even where transcription was not detected, and transcription was not detected in any case where we had predicted that the target would be absent. However, in some cases, predicted differentiating genes did not appear to be transcribed under the conditions of the experiment, as the predicted amplicon was not observed even though the gene was detected by PCR in the genomic DNA. All of the identified differentials have been used in development of a Brucella microarray containing probes for both common and differentiating targets.

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